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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
HILL, KEVIN KAI	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/551,876	CONDIE ET AL.	
	Examiner	Art Unit	
	KEVIN K. HILL	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03 March 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *46,50-53 and 55-68* is/are pending in the application.

4a) Of the above claim(s) *52,53,58 and 59* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *46,50,51,55-57 and 60-68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *March 3, 2008*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

Election/Restrictions

Applicant's response to the Requirement for Restriction, filed on July 30, 2007, is acknowledged.

Applicant has elected the invention of Group XI, Claims 46 and 48-60, drawn to a method of stabilizing pluripotent stem cells, the method comprising contacting the pluripotent cell culture with an inhibitor of at least one component of the gamma secretase complex.

Within Group XI, Applicant has elected the Notch inhibitor species "DAPT", an inhibitor of the gamma secretase complex, regarding Claim 60.

In a telephone conversation with Applicant's representative, Kathryn Wade at 404-854-8000, on September 20, 2007, Applicant has elected the pluripotent cell species human embryonic stem cells.

Amendments

Applicant's response and amendments, filed March 3, 2008, to the prior Office Action is acknowledged. Applicant has cancelled Claims 1-45, 47-49 and 54, withdrawn Claims 52-53 and 58-59, amended Claims 46, 50-51, 55-57 and 60, and added new claims, Claims 61-68.

Claims 52-53 and 58-59 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim.

Claims 46, 50-51, 55-57 and 60-68 are under consideration.

Priority

This application is a 371 of PCT/US04/09817, filed March 31, 2004 which claims benefit of a prior-filed parent provisional application 60/459,129, filed on March 31, 2003 and claims benefit of provisional application 60/516,582 filed October 31, 2003 under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged.

Accordingly, the effective priority date of the instant application is granted as March 31, 2003.

Information Disclosure Statement

Applicant has filed an Information Disclosure Statement on March 3, 2008 that has been considered. The signed and initialed PTO Form 1449 is mailed with this action.

Examiner's Note

Unless otherwise indicated, previous objections/rejections that have been rendered moot in view of the amendment will not be reiterated. The arguments in the March 3, 2008 response will be addressed to the extent that they apply to current rejection(s).

Specification

1. **The objection to the disclosure is withdrawn** in light of Applicant's amendment to the specification in the papers filed March 3, 2008 to remove the embedded hyperlinks.

Claim Objections

2. **The prior objections to Claims 51 and 54 are withdrawn** in light of Applicant's amendment to Claim 51 reciting the complete name of DAPT before using its acronym, and for cancelling duplicate claim 54.

3. **Claims 62-68 are objected to because of the following informalities**:

Applicant is advised that should Claims 46, 51, 55-57 and 60-61 be found allowable, Claims 62-68 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). In the instant case, independent Claims 46 and 62 differ only in the terms describing the result of administering DAPT to the human embryonic stem cell culture, to wit "maintaining...in an undifferentiated state" vs. "inhibiting differentiation". However, the specification discloses that "maintaining" is understood to mean that the cells will proliferate over multiple passages yielding cells of the same differentiation state, wherein the "maintained" cell does not further differentiate (pg 16, [051]). Thus, the method of maintaining hESCs in an undifferentiated state (claim 46) inherently achieves the method of inhibiting differentiation (claim 62), and vice versa.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the Applicant regards as his invention.

4. **The prior rejection of Claims 46, 48-51, 54-57 and 60 are rejected under 35 U.S.C. 112, second paragraph, is withdrawn** in light of Applicant's amendments to the claims to clarify that the method maintains the embryonic stem cell in an undifferentiated state, and for cancelling the term "non-transition state analogue".

5. **Claim 51 is rejected under 35 U.S.C. 112, second paragraph**, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

This is a new rejection.

The claim recites a γ-secretase inhibitor comprising DAPT. However, the claim is indefinite because it does not recite the structural features of compound. For example, if the core DAPT structure has been further chemically modified, what is that structure, and is the new compound still a γ-secretase inhibitor? It would be remedial to replace "comprises" with "is". (See Claim 63, for example.)

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. **Claims 46, 50-51, 55-57, 60, 62 and 64-67 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement.** The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

This is a new rejection.

The claimed invention is directed to a method inhibiting the differentiation of human embryonic stem cells comprising the administration of an inhibitor of at least one component of the γ-secretase complex. At issue for the purpose of written description requirements is the

enormous breadth of the genus of structurally distinct compounds capable of inhibiting at least one component of the γ-secretase complex.

> *Vas-cath Inc. v. Mahurkar*, 19USPQ2d 1111, clearly states that Applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." (See page 1117.) The specification should "clearly allow persons of ordinary skill in the art to recognize that (he or she) invented what is claimed." (See *Vas-cath* at page 1116).

In analyzing whether the written description requirement is met for genus claims, it is first determined whether a representative number of species have been described by their complete structure. The disclosure of a single species is rarely, if ever, sufficient to describe a broad genus, particularly when the specification fails to describe the features of that genus, even in passing. (see *In re Shokal* 113USPQ283(CCPA1957); *Purdue Pharma L.P. vs Faulding Inc.* 56 USPQ2nd 1481 (CAFC 2000). In the instant case, the specification discloses a plurality of compounds contemplated to inhibit at least one component of the γ-secretase complex, i.e. DAPT, transition state analogues, helical peptides containing α-aminoisobutyric acid, Fenchylamine Sulfonamide compounds, NSAIDs and benzodiazepines (pg 12, [040].

Next, then, it is determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics (i.e. other than nucleotide sequence), specific features and functional attributes that would distinguish different members of the claimed genus. To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of compete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, methods of making the claimed product, or any combination thereof. However, in the instant case, the plurality of compounds [040] do not share a common structural feature that would adequately represent the breadth of the claimed genus of γ-secretase inhibitors and clearly inform an artisan that a particular agent would necessarily possess the property of inhibiting γ-secretase.

It is noted that all these compounds, NSAIDs, benzodiazepines, transition state analogues, Fenchylamine Sulfonamide compounds, etc… vary greatly in structure and function and therefore each represents a subgenus. Again, the members of any of the subgenuses themselves would have very different structure and the specification does not provide any description of any identifying characteristics of the species of the subgenuses.

The art recognizes that the γ-secretase is a high molecular weight, multi-protein complex comprising at least five different proteins: Presenilin-1, Presenilin-2, Nicastrin, Aph1 and Pen-2. At the time of the invention, Geling et al (2002: *of record) taught that the nature of γ-secretase is still unclear. Two homologous presenilin proteins, PS1 and PS2, may be aspartyl proteases with gamma-secretase activity. However, additional co-factors are required to allow formation of the biologically active PS complex, and the cellular distribution of PS does not necessarily reflect the location of gamma-secretase activity (pg 688, col. 2).Similarly, Tian et al (J. Biol. Chem. 278(31):28968-28975, 2003) teach that the identity and structure of γ-secretase remains elusive, and its kinetic and catalytic mechanisms are poorly understood. To a large extent, this is due to the highly complicated structural organization of this unusual protease. The precise roles of each protein subunit in the catalytic mechanism of γ-secretase awaits further investigation (pg 28968, col. 2). The kinetics and mode of action by which each compound capable of inhibiting γ-secretase is not readily predictable, and thus the working concentration of one inhibitor species would not be readily extrapolated to the working concentration of another inhibitor species (Tian et al; Kornilova et al, J. Biol. Chem. 278(19):16470-16473, 2003).

Furthermore, γ-secretase inhibitors inhibit membrane cleavage of several developmentally important signal transduction molecules, including N-cadherin, E-cadherin, ErbB4, CD44 and Notch (Tian et al, pg 28968, col. 2; Lowell et al, PLOS Biology 4(5):e121, 2006; pg 809, col. 1). The mechanism(s) by which γ-secretase reacts with these different substrates remains unknown, and thus the genus of claimed inhibitors are not pathway-specific inhibitors that would result in a predictable cellular response. Generally, substrate specificity of an enzyme cannot be modified by affecting catalysis alone since all substrates access the same catalytic machinery of the enzyme. For enzymes with substrate binding and catalysis at the same site, the only way of modulating substrate specificity is by modifying the affinity for different substrates. For enzymes with separated substrate binding and catalytic sites, substrate specificity

may also be controlled by affecting substrate movement, if such a motion is substrate-specific. This is straightforward conceptually if multiple substrate binding sites exist, a concept that remains to be tested directly. It is a bit more difficult to conceptualize whether only a single substrate binding site exists, although still feasible in principle depending on the higher order structural features associated with the movement of different substrates into the catalytic site (Tian et al, pg 28974, col. 1).

The claims recite that the administration of an enormous genus of structurally distinct γ-secretase inhibitors to human embryonic stem cells will result in the inhibition of differentiation. However, the post-filing art teaches that not all γ-secretase inhibitors possess this functional property. For example, the γ-secretase inhibitor L-685,458 is an effective inhibitor of Notch activation. However, if the inhibitor (Lowell et al; used at 4μm; pg 815, col. 2, Pharmacological) is maintained beyond 5 days, the embryonic stem cells progressively begin to differentiate into a non-neural flattened morphology. Lowell et al's observations suggest that blockade of γ-secretase initially delays lineage commitment and then diverts ES cells into a non-neural fate.

In the instant case, the specification only discloses the use of DAPT at a concentration of 50μM DAPT for three days prior to EDTA treatment (pg 56, [0163]). Under such conditions, NICD fragment was not generated in BGN1 cultures. The specification does not disclose a nexus between the use of 50μM DAPT for three days prior to EDTA treatment and the necessary concentration of the enormous genus of structurally distinct compounds to inhibit the differentiation of human embryonic stem cells.

The Revised Interim Guidelines state:

"The claimed invention as a whole may not be adequately described if the claims require an essential or critical element which is not adequately described in the specification and which is not conventional in the art" (col. 3, page 71434), "when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus", "in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus" (col. 2, page 71436).

An Applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

Possession may also be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the Applicant was in possession of the claimed invention. See, e.g., *Pfaff v. Wells Elecs., Inc.*, 525 U.S. 55, 68, 1 19 S.Ct. 304, 312, 48 USPQ2d 1641, 1647 (1998), *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997)*, *Amgen, Inc. v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) (one must define a compound by "whatever characteristics sufficiently distinguish it").

Therefore, conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. See *Fiers v. Revel*, 25 USPQ2d 1602 at 1606 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016. One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481, 1483. *In Fiddes*, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Without a correlation between structure and function, the claim does little more than define the claimed invention by function. That is not sufficient to satisfy the written description requirement. *See Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 ("definition by function … does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is").

Based on the Applicant's specification, the skilled artisan cannot envision the detailed chemical structure of the enormous genus of γ-secretase inhibitors reasonably encompassed by the claims, and the necessary working concentration of each inhibitor so as to perform the claimed method and achieve inhibition of differentiation of human embryonic stem cells. The one species of agent specifically disclosed, DAPT, is not representative of the genus because the genus is highly variant, the structural identity and mechanism of action of γ-secretase is unknown and poorly understood, and the kinetics of one γ-secretase inhibitor does not establish the kinetics of another γ-secretase inhibitor.

Accordingly, given that the specification does not teach a common structural feature that would identify a compound to be a γ-secretase inhibitor, nor the necessary amount to inhibit the differentiation of human embryonic stem cells, this limited information is not deemed sufficient to reasonably convey to one skilled in the art that the Applicant is in possession of the required starting materials, that is the broad genus of γ-secretase inhibitors, to perform the necessary active steps and effect the claimed method, at the time the application was filed.

Thus, for the reasons outlined above, it is concluded that the claims do not meet the requirements for written description under 35 U.S.C. 112, first paragraph. Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

7. **Claims 46, 50-51, 55-57 and 60 stand, and Claims 61-62 and 64-67 are newly rejected under 35 U.S.C. 112, first paragraph** as failing to comply with the enablement requirement. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

The rejection of the prior Office Action has been modified for the presentation of new arguments. Thus, this is considered a new rejection.

At issue for the enablement requirement is that the specification does not disclose the culture conditions to predictably use the enormous genus of structurally undisclosed compounds capable of inhibiting the gamma secretase complex to maintain the hES cells in an undifferentiated state.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention. If not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets

the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2ds 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification. Therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention. And thus, skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The Breadth of the Claims and The Nature of the Invention

The claims are broad for encompassing a genus of structurally distinct compounds that possess distinctly different biochemical and mechanistic properties that are to be used alone or in combination for the treatment of pluripotent cells. The breadth of the claim reasonably encompasses an enormous genus of structurally undisclosed compounds capable of inhibiting at least one component of the gamma secretase complex, wherein any given species of the genus may be used at an essentially infinite possible concentration.

The inventive concept of the instant application is that the administration of DAPT, a γ-secretase inhibitor, at a concentration of 50μM will maintain human embryonic stem cells in an undifferentiated state, at least in short-term cultures.

The Existence of Working Examples and The Amount of Direction Provided by the Inventor

The specification discloses the culture of human BGN1 embryonic stem cells maintained on mouse embryonic fibroblasts (pg 47, [0137]). After a degree of manual selection of cells possessing "good cellular morphology" and manual cell passaging, a small number of colonies presented a phenotype of "compact dome morphology" that was stabilized for more than 20 passages (pg 49, [0142]). Magnetic sorting for SSEA4 expression enriched for the compact dome morphology, wherein said cells express the Oct-4, SSEA3, TRA-1-60, TRA-1-81 and Notch-1 markers (pg 49, [0143-0144]).

Enzymatic methods to culture and transfer small clumps of hES cells employ the use of trypsin/EDTA to break hES cells down to an essentially single cells suspension for transfer to fresh dishes (pg 54, [0157]). EDTA exposure can activate the γ-secretase complex in hES cells to cleave Notch (pg 56, [0162-0163]). Notch-1 (non-cleaved) is highly expressed on the surface of morphologically undifferentiated hES cells; whereas, differentiating cells are negative for Notch-1 (pg 7, [022]; pg 8, [025]). However, Oct-4, an indicator of undifferentiated state, is expressed in both EDTA-treated (Notch-activated) and EDTA-untreated (Notch-inactivated) samples (pg 56, [0162]), and thus no strict correlation between Notch activation/inactivation and the undifferentiated state exists.

Human ES cells were grown in 50μM DAPT, a γ-secretase inhibitor, for three days prior to EDTA treatment (pg 56, [0163]). Under such conditions, NICD fragment was not generated in BGN1 cultures.

Applicant has discovered that inhibition of γ-secretase reduces the number of spontaneously differentiated cells in hES cell culture and maintains [stabilizes] their pluripotent

phenotype when DAPT is used at a concentration of 50μM (pg 57, [0165]). DAPT treatment of trypsin-passaged cultures returned embryoid bodies having a non-cystic phenotype to embryoid bodies having a cystic phenotype. Therefore, inhibition of gamma-secretase appeared to reduce the number and proportion of differentiating cells in hES cultures, thereby leading to an increase in homogeneity of the cultures (pg 58, [0167-0168]).

At issue for the enablement requirement is that the specification does not disclose the culture conditions to predictably use the enormous genus of structurally undisclosed compounds capable of inhibiting the gamma secretase complex to maintain the hES cells in an undifferentiated state. While the specification discloses the passaging of hES cells for more than 40 passages (pg 57, Example 5), the working example does not disclose the DAPT treatment condition variables necessary to achieve the reduced number of spontaneously differentiated cells, such as duration, frequency or application before or after critical steps necessary to passage the cells.

The State of the Prior Art and The Level of One of Ordinary Skill

The Gamma-Secretase Complex and DAPT

The art recognizes that the γ-secretase is a high molecular weight, multi-protein complex comprising at least five different proteins: Presenilin-1, Presenilin-2, Nicastrin, Aph1 and Pen-2. At the time of the invention, Geling et al (EMBO Reports 3(7):688-694, 2002) taught that the nature of γ-secretase is still unclear. Two homologous presenilin proteins, PS1 and PS2, may be aspartyl proteases with gamma-secretase activity. However, additional co-factors are required to allow formation of the biologically active PS complex, and the cellular distribution of PS does not necessarily reflect the location of gamma-secretase activity (pg 688, col. 2). Similarly, Tian et al (J. Biol. Chem. 278(31):28968-28975, 2003) teach that the identity and structure of γ-secretase remains elusive, and its kinetic and catalytic mechanisms are poorly understood. To a large extent, this is due to the highly complicated structural organization of this unusual protease. The precise roles of each protein subunit in the catalytic mechanism of γ-secretase awaits further investigation (pg 28968, col. 2). The kinetics and mode of action by which each compound capable of inhibiting γ-secretase is not readily predictable, and thus the working concentration of one inhibitor species would not be readily extrapolated to the working concentration of another inhibitor species (Tian et al; Kornilova et al, J.Biol. Chem. 278(19):16470-16473, 2003).

Furthermore, γ-secretase inhibitors inhibit membrane cleavage of several developmentally important signal transduction molecules, including N-cadherin, E-cadherin, ErbB4, CD44 and Notch (Tian et al, pg 28968, col. 2; Lowell et al, PLOS Biology 4(5):805-818,

2006; pg 809, col. 1). The mechanism(s) by which γ-secretase reacts with these different substrates remains unknown, and thus the genus of claimed inhibitors are not pathway-specific inhibitors that would result in a predictable cellular response. Generally, substrate specificity of an enzyme cannot be modified by affecting catalysis alone since all substrates access the same catalytic machinery of the enzyme. For enzymes with substrate binding and catalysis at the same site, the only way of modulating substrate specificity is by modifying the affinity for different substrates. For enzymes with separated substrate binding and catalytic sites, substrate specificity may also be controlled by affecting substrate movement, if such a motion is substrate-specific. This is straightforward conceptually if multiple substrate binding sites exist, a concept that remains to be tested directly. It is a bit more difficult to conceptualize whether only a single substrate binding site exists, although still feasible in principle depending on the higher order structural features associated with the movement of different substrates into the catalytic site (Tian et al, pg 28974, col. 1).

The claims recite that the administration of an enormous genus of structurally distinct γ-secretase inhibitors to human embryonic stem cells will result in the inhibition of differentiation. DAPT (CAS # 208255-80-5) is an art-recognized γ-secretase inhibitor (Dovey et al, J. Neurochem. 76: 173-181, 2001; *of record in IDS). However, the post-filing art teaches that not all γ-secretase inhibitors possess this functional property. For example, the γ-secretase inhibitor L-685,458 is an effective inhibitor of Notch activation. If the inhibitor (Lowell et al; used at 4μm; pg 815, col. 2, Pharmacological) is maintained beyond 5 days, the embryonic stem cells progressively begin to differentiate into a non-neural flattened morphology. Lowell et al's observations suggest that blockade of γ-secretase initially delays lineage commitment and then diverts ES cells into a non-neural fate.

In the instant case, the specification only discloses the use of DAPT at a concentration of 50μM DAPT for three days prior to EDTA treatment (pg 56, [0163]). Under such conditions, NICD fragment was not generated in BGN1 cultures. The specification does not disclose a nexus between the use of 50μM DAPT for three days prior to EDTA treatment and the necessary concentration of the enormous genus of structurally distinct compounds to inhibit the differentiation of human embryonic stem cells.

The Level of Predictability in the Art

"[I]t is fair to say that the science of pluripotent stem cells is still in its infancy." (Verfillie et al, pg 375, col. 1, lines 12-14). So far, there has been no report of large-scale expansion of undifferentiated hES cells principally, because the basic biological knowledge of factors that maintain hES pluripotency is still being developed (Ulloa, pg 23, col. 1, ¶2). Considerable progress has been made toward the generation of more defined hES cell culture conditions since initial isolation and growth conditions were described. However, current hES cell culture conditions need to be improved. The optimal culture conditions will be defined by the goals of the investigators and the use of the cells. Although considerable progress has been made toward these goals, there is still a significant challenge ahead (Hoffman et al; pg 706, Future Perspectives). It will be important to identify factors that facilitate growth and inhibit differentiation of human ES cells (Pera et al, pg 9, col. 1, ¶2). Thus, the art recognizes considerable unpredictability in methods of maintaining embryonic stem cells in an undifferentiated state.

In particular regards to the instant application, the art teaches that Notch activity is required at different times in ES cell culture to either maintain the undifferentiated state or to promote the differentiation of the ES cells. However, the timing and robustness of Notch signaling relative to other important signal transduction pathways is complex and not fully understood. This lack of understanding regarding the precise role and degree of Notch signaling is compounded by the poorly understood nature of the γ-secretase complex and its mechanism of action. Tian et al (J. Biol. Chem. 278(31):28968-28975, 2003) teach that the identity and structure of γ-secretase remains elusive, and its kinetic and catalytic mechanisms are poorly understood. To a large extent, this is due to the highly complicated structural organization of this unusual protease. The precise roles of each protein subunit in the catalytic mechanism of γ-secretase awaits further investigation (pg 28968, col. 2). The kinetics and mode of action by which each compound capable of inhibiting γ-secretase is not readily predictable, and thus the working concentration of one inhibitor species would not be readily extrapolated to the working concentration of another inhibitor species (Tian et al; Kornilova et al, J.Biol. Chem. 278(19):16470-16473, 2003).

The Quantity of Any Necessary Experimentation to Make or Use the Invention

Thus, the quantity of necessary experimentation to make or use the invention as claimed, based upon what is known in the art and what has been disclosed in the specification, will create an undue burden for a person of ordinary skill in the art to demonstrate that the administration of a genus of undisclosed and structurally distinct compounds capable of inhibiting at least one

component of the γ-secretase complex will maintain human embryonic stem cells in an undifferentiated state. The art recognizes considerable variance in embryonic stem cell culture protocols and the ability to passage such cell long term while maintaining the cells in an undifferentiated state because said cells regularly spontaneously differentiate. The instant specification does not disclose a nexus between the specific 50μM DAPT condition and the necessary concentration(s) of the enormous genus of structurally distinct compounds capable of inhibiting at least one component of the γ-secretase complex necessary to achieve the reduced number of spontaneously differentiated cells, such as duration, frequency or application before or after critical steps necessary to passage the cells. Such specific disclosure is necessary because the art recognizes that the kinetics and mechanism of action of γ-secretase is poorly understood, the activity of one γ-secretase inhibitor does not predictably teach the use of another structurally distinct γ-secretase inhibitor, that Notch signaling, in and of itself as well as in relation to other critical signal transduction pathways, is complex, and teaches a requirement for Notch signaling to maintain the undifferentiated state that is counter to the instant specification.

Accordingly, the instant claims are rejected for failing to comply with the enablement requirement.

Claim Rejections - 35 USC § 102

8. **The prior rejection of Claims 46 and 60 under 35 U.S.C. 102(b)** as being anticipated by Karanu et al (J. Exp. Med 192(9): 1365-1372, 2000), as evidenced by Small et al (J. Biol. Chem. 276(34):32022-32030, 2001) **is withdrawn** in light of Applicant's amendment to the claims, limiting the scope of the cells to human embryonic stem cells, which Karanu et al do not teach.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the Examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any

evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the Examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. **The prior rejection of Claims 46, 48-49 and 60 under 35 U.S.C. 103(a)** as being unpatentable over Karanu et al (2000; *of record), as evidenced by Small et al (J. Biol. Chem. 276(34):32022-32030, 2001) and Walsh et al (Acta Pathological, Microbiologica et Immunologica Scandinavica (APMIS) 111(1):197-210, 2003) **is withdrawn** in light of Applicant's arguments that Walsh et al teach away from the claimed invention, because Walsh et al. describe that activating Notch signaling enhances ES cell self-renewal, contrary to the teaching of the present specification, which shows that inhibiting gamma secretase (which inhibits notch signaling, among other things) enhances ES cell self renewal, which the Examiner finds to be persuasive.

10. **The prior rejection of Claims 46, 50-51, 54 and 55-57 under 35 U.S.C. 103(a)** as being unpatentable over Karanu et al (J. Exp. Med 192(9): 1365-1372, 2000), as evidenced by Small et al (J. Biol. Chem. 276(34):32022-32030, 2001) and Walsh et al (Acta Pathological, Microbiologica et Immunologica Scandinavica (APMIS) 111(1):197-210, 2003), as applied to claims 46, 48-49 and 60 above, and in further view of Dovey et al (J. Neurochem. 76: 173-181, 2001; *of record in IDS), as evidenced by Pera et al (J. Cell Science 113: 5-10, 2000) and Nakhei et al (Nucleic Acids Res. 26(2): 497-504, 1998) **is withdrawn** in light of Applicant's arguments as discussed above.

11. **Claims 46, 50-51, 60-63 and 67-68 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Karanu et al (2000; *of record) in view of Ema et al (U.S. 2005/0221477), Geling et al (2002; *of record), as evidenced by Small et al (2001; *of record).

This is a new rejection.

Determining the scope and contents of the prior art.

Karanu et al teach a method of maintaining the survival and expansion of human hematopoietic stem cells in an undifferentiated state in cell culture, the method comprising the administration of soluble human Jagged-1 extracellular domain (pg 1366, col. 1, hJagged-1 protein; pg 1369, Figure 3), wherein the art recognizes soluble human Jagged-1 extracellular domain to be an inhibitor of Notch signaling (Small et al; pgs 32026-32027, joining ¶). Karanu et al teach that the activity of soluble human Jagged-1 extracellular domain provides an opportunity

for the optimization for clinical protocols aimed at *ex vivo* expansion of human stem cells (pg 1366, col. 1).

Karanu et al do not teach the human stem cells to be human embryonic stem cells. However, at the time of the invention, Ema et al disclosed a method of sustaining stem cells in an undifferentiated state and maintaining pluripotentcy comprising the step of providing a polypeptide having a WIF-1 EGF-like motif, e.g. Jagged-1 and Dlk [0165, 0232]. Thus, it becomes possible to easily provide a large amount of stem cells, wherein Ema et al contemplate such stem cells as hematopoietic stem cells and human embryonic stem cells [0150], wherein such polypeptides maintain pluripotentcy with blocking or delaying differentiation (i.e. maintaining the undifferentiated state). Ema et al disclose a range of concentrations for which the artisan may apply a given polypeptide to a stem cell culture [0233], demonstrating that it is well within the skill of the ordinary artisan to change concentrations of the active agent so as to optimize and achieve the desired effect.

Neither Karanu et al nor Ema et al teach the Notch inhibitor to be an inhibitor of at least one component of the gamma-secretase complex, specifically DAPT. However, at the time of the invention, Geling et al taught that DAPT, an art-recognized inhibitor of at least one component of the gamma secretase complex, is also an inhibitor of Notch signaling.

Ascertaining the differences between the prior art and the claims at issue, and Resolving the level of ordinary skill in the pertinent art.

People of the ordinary skill in the art will be highly educated individuals such as medical doctors, scientists, or engineers possessing advanced degrees, including M.D.'s and Ph.D.'s. Thus, these people most likely will be knowledgeable and well-read in the relevant literature and have the practical experience in preparing and passaging embryonic stem cells, and knowledge of the important cell signal transduction pathways affecting cellular differentiation. Therefore, the level of ordinary skill in this art is high.

Neither Karanu et al, Ema et al nor Geling et al teach the use of DAPT at a concentration of 50μM. However, Ema et al disclose that the active agent that inhibits differentiation of

embryonic stem cells may be used at a range of concentrations [0233], and thus, absent evidence to the contrary, it is well within the skill of the ordinary artisan to vary the concentration of the active agent so as to achieve the desired effect, e.g. 50μM.

Considering objective evidence present in the application indicating obviousness or nonobviousness.

It would have been obvious to one of ordinary skill in the art to substitute the hematopoietic stem cells as taught by Karanu et al with human embryonic stem cells as taught by Ema et al in a method of maintaining the survival and expansion of human stem cells in an undifferentiated state in cell culture with a reasonable chance of success because the simple substitution of one known element for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention. Those of ordinary skill in the art recognize that hematopoietic stem cells and embryonic stem cells are but species within the same genus of stem cells (Ema et al). An artisan would be motivated to substitute hematopoietic stem cells with human embryonic stem cells in a method of maintaining the survival and expansion of human stem cells in an undifferentiated state in cell culture because hematopoietic stem cells exist at a later differentiation stage and are limited in their developmental potential; whereas, embryonic stem cells are totipotent, and thereby have the possibility of differentiating into any cell type.

It would have been obvious to one of ordinary skill in the art to substitute a first Notch inhibitor, e.g. soluble Jagged 1 as taught by Karanu et al, with another Notch inhibitor, e.g. DAPT as taught by Geling et al, in a method of maintaining the survival and expansion of human stem cells in an undifferentiated state in cell culture with a reasonable chance of success because the simple substitution of one known element for another would have yielded predictable results to one of ordinary skill in the art at the time of the invention. An artisan would be motivated to substitute a first Notch inhibitor, e.g. soluble Jagged 1, with another Notch inhibitor, e.g. DAPT, in a method of maintaining the survival and expansion of human stem cells in an undifferentiated state in cell culture because DAPT is a chemical compound; whereas, soluble Jagged 1 is a polypeptide, and thus it would be easier for the artisan to acquire, store and work with DAPT than a soluble polypeptide whose biological activity would be strongly dependent upon the method of manufacture, stability in solution, and subject to degradation, e.g. lot-to-lot variation.

Thus, absent evidence to the contrary, the invention as a whole is *prima facie* obvious.

12. **Claims 55-57 and 64-66 are rejected under 35 U.S.C. 103(a)** as being unpatentable over Karanu et al (2000; *of record) in view of **Ema et al (U.S. 2005/0221477),** Geling et al (2002; *of record), as evidenced by Small et al (2001; *of record), as applied to Claims 46, 50-51, 60-63 and 67-68 above, and in further view of Walsh et al (2003; *of record), Pera et al (2000; *of record) and Nakhei et al (1998; *of record).

This is a new rejection.

Determining the scope and contents of the prior art.

Neither Karanu et al, Ema et al nor Geling et al teach wherein the differentiation is inhibited for at least 10 passages, wherein the differentiation state is determined by expression of SSEA4 and Notch1 in at least 60% of the cells, and wherein less than about 20% of the hESCs express HNF4α after about 10 passages. However, at the time of the invention,

However, absent evidence to the contrary, those of ordinary skill in the art would recognize that such limitations would naturally flow when embryonic stem cells are passaged in the presence of an inhibitor of Notch signaling, specifically DAPT.

For example, the prior art recognized that human embryonic stem cells express Notch 1 (Walsh et al) and SSEA-4 (Pera et al; pg 8, Table 1), and that HNF4α is a tissue-specific transcription factor mainly expressed in endodermal tissues such as liver, kidney, intestine and endocrine pancreas, thus indicating differentiation of stem cells towards endodermal tissues (Nakhei et al). Thus, a human embryonic stem cell population passaged in the presence of an inhibitor of Notch signaling, specifically DAPT, will necessarily express the recited markers in the recited proportions for the recited number of passages because an inhibitor of Notch signaling is expected to maintain the survival and expansion of stem cells in an undifferentiated state.

Ascertaining the differences between the prior art and the claims at issue, and Resolving the level of ordinary skill in the pertinent art.

People of the ordinary skill in the art will be highly educated individuals such as medical doctors, scientists, or engineers possessing advanced degrees, including M.D.'s and Ph.D.'s. Thus, these people most likely will be knowledgeable and well-read in the relevant literature and have the practical experience in preparing and passaging embryonic stem cells, and knowledge of the important cell signal transduction pathways affecting cellular differentiation. Therefore, the level of ordinary skill in this art is high.

The cited prior art does not teach the step of determining the level of marker expression at or around passage 10. However, absent evidence to the contrary, nothing non-obvious is seen with performing a marker expression level determination step at or around passage 10 because it is well within the skill of the artisan to ascertain the expression level of a marker at any passage.

Considering objective evidence present in the application indicating obviousness or nonobviousness.

It would have been obvious to one of ordinary skill in the art to combine the steps of determining the expression of SSEA4, Notch1 and HNF4α in a method of maintaining the survival and expansion of human stem cells in an undifferentiated state in cell culture because "a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipate success, it is likely that product not of innovation but of ordinary skill and common sense." An artisan would be motivated to combine the steps of determining the expression of SSEA4, Notch1 and HNF4α in a method of maintaining the survival and expansion of human stem cells in an undifferentiated state in cell culture because those of ordinary skill in the art recognized that SSEA4 is a marker for totipotent human embryonic stem cells, Notch1 expression levels decrease as the embryonic stem cells differentiate, and HNFα is a marker for differentiation, and thus the artisan would be able to measure the proportion of cells in the population that remain in an undifferentiated state as compared to those cells that have begun to differentiate. Based upon this result, the artisan may desire to increase or decrease the effective concentration of the Notch inhibitor to optimize the yield of undifferentiated human embryonic stem cells at each passage of the cell culture.

Thus, absent evidence to the contrary, the invention as a whole is *prima facie* obvious.

Conclusion

13. No claims are allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Kevin K. Hill, Ph.D. whose telephone number is 571-272-8036. The Examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Joseph T. Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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Examiner, Art Unit 1633

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